

Q1:

You want to have the spoVM protein produced by *E. coli* cells, because you are interested in purifying the protein and using it for the production of glue. Below, a fragment of the cloning vector pUC19 can be seen (pUC19 is in total 2,686 bp) in which the gene encoding spoVM is inserted (the green nucleotides). Please note that only one of the strands is shown – the other one you can write yourself, if you'd like...

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>pUC19 [length=2686] [version=09-MAY-2008] [topology=circular] Cloning vector
pUC19, complete sequence.
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60
5' TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCA
3' AGCGCGCAAAGCCACTACTGCCACTTTTGGAGACTGTGTACGTCGAGGGCCTCTGCCAGT

120
CAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTG
GTCGAACAGACATTTCGCTACGGCCCTCGTCTGTTTCGGGCAGTCCCGCGCAGTCGCCCAC

180
TTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGC
AACCGCCACAGCCCCGACCGAATTGATACGCCGTAGTCTCGTCTAACATGACTCTCACG

240
ACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCC
TGGTATACGCCACA...osv...osv...osv...

300
ATTCGCCATTTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTAT

360
TACGCCAGCTGGCGAAAGGGGATGTGCTGCAAGGCGATTAAAGTTGGGTAACGCCAGGGTT

420
TTCCCAGTCACGACGTTGTAAACGACGGCCAGTGAATTGAGCTCGGTACCCGATGAAAT

480
TTTATACAATCAAACAGCTAGATTTCTTGGCGGCATTGTCCGTGCGATGCTTGGTTTCAAT

540
TTAAGAAAGATTAAAGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCGTAATC

600
TGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGA

660
GCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATT

GCGTTGCGCTCACTGCCCCGCTTTCAGTCGGGAAACCTGTGCTGCGCAGCTGCATTA 3'
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Since you want to make sure that the gene is successfully inserted in the vector, you want to perform PCR using the below primers:

Primer A: 5' AGTGAATTGAGCTCGGTACC 3'

Primer B: 3' AGGAGATCTCAGCTGGACGT 5'

The nucleotides to which the primers bind are underlined in the sequence above. Note, however, that primer A will bind to the DNA strand that is not shown (but which you may have written yourself?) Answer the below questions:

a. How large will the PCR product be, if the *spoVM* gene is successfully inserted?

Answer: If the *spoVM* gene is successfully inserted, the PCR product will be 127 bp (each primer is 20 bp and the area between the two primers is 87 bp: $2 * 20 + 87 = 127$)

b. How large will the PCR product be, if the *spoVM* gene has not been inserted?

Answer: If the *spoVM* gene has not been inserted, the PCR product will be 46 bp (the *spoVM* gene is 81 bp (the green bases): $127 - 81 = 46$).

Instead of primer B, you try to use primer C with the following sequence:

Primer C: 5' TGCAGGTCGACTCTAGAGGA 3'

c. How large will the PCR product be, if you use primer A and C and the *spoVM* gene is successfully inserted?

Answer: This was a trick-question! Primer C is identical to primer B, just written from the other end. Accordingly, the PCR product will still be 127 bp as in a.

d. How large will the PCR product be, if you use primer A and C and the *spoVM* gene has not been inserted?

Answer: 46 bp (as in b).

Q2:

Since women were allowed in the Olympics in 1912, it has been a concern that male athletes would impose as women and thereby have an unfair advantage in the female competitions. One of the tests

performed to ensure that the athletes competing as women *were* actually women, was based on detection of the *SRY* gene, which is placed on the Y chromosome. Below, the sequence of the *SRY* gene is shown.

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>gi|157310125:c615-1 Homo sapiens SRY gene for sex determining region Y,
isolate ADT3

70
5' ATGCAATCATATGCTTCTGCTATGTTAAGCGTACTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAGA
3' TACGTTAGTATACGAAGACGATACAATTTCGCATGAGTTGTCGCTACTAATGTCAGGTCGACACGTTCTCT

140
ATATTCCCGCTCTCCGGAGAAGCTCTTCCTTCCTTTGCACTGAAAGCTGTAAGTCTAAGTATCAGTGTGA
TATAAGGGCGAGAGGCCCTCTTCGAGAAGGAAGGAAACGTGACTTTCGACATTGAGATTCATAGTCACACT
Here primer A binds

210
AACGGGAGAAAACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTTCATCGTGTGG
TTGCCCTCTTTTGTCAATTCGGTTGCAGGTCCTATCTCACTTCGCTGGGTACTTGCGTAAGTAGCACACC

Here primer B binds

280
TCTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCCAGAATGCGAACTCAGAGATCAGCAAGCAGC
AGAGCGCTAGTCTCCGCGTTCTACCGAGATCTCTTAGGGTCTTACGCTTTGAGTCTCTAGTCGTTTCGTCG

350
TGGGATACCAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCCAGGAGGCACAGAAATTACA
ACCCTATGGTCACCTTTTACGAATGACTTCGGCTTTTTACCGGTAAGAAGGTCCTCCGTGTCTTTAATGT

420
GGCCATGCACAGAGAGAAATACCCGAATTATAAGTATCGACCTCGTCGGAAGGCGAAGATGCTGCCGAAG
CCGGTACGTGTCTCTCTTTATGGGCTTAATATTCATAGCTGGAGCAGCCTTCCGCTTCTACGACGGCTTC

490
AATTGCAGTTTGCTTCCCGCAGATCCCGCTTCGGTACTCTGCAGCGAAGTGCAACTGGACAACAGGTTGT
TTAACGTCAAACGAAGGGCGTCTAGGGCGAAGCCATGAGACGTCGCTTCACGTTGACCTGTTGTCCAACA

560
ACAGGGATGACTGTACGAAAGCCACACACTCAAGAATGGAGCACCAGCTAGGCCACTTACCGCCCATCAA
TGTCCTACTGACATGCTTTCGGTGTGTGAGTTCTTACCTCGTGGTCGATCCGGTGAATGGCGGGTAGTT

CGCAGCCAGCTCACCGCAGCAACGGGACCGCTACAGCCACTGGACAAAGCTGTAG 3'
CGGTCGGTCGAGTGGCGTCGTTGCCCTGGCGATGTCGGTGACCTGTTTCGACATC 5'
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Describe how you can use PCR to examine whether a person is a man or a woman. Make sure that you include the sequence of the two primers that you use to amplify a section of the *SRY* gene. The section that you aim at amplifying should be approximately 200 bp long. Also remember to mark the 3' and 5' ends of the primers and where they bind to the *SRY* gene.

Answer: For PCR you need DNA, which in this case should be purified from cells of the athlete. Furthermore, you need two primers that are specific for the *SRY* gene. The two primers could **for**

instance be primer A and B, the sequence of which is shown below. It is indicated on the figure above where the two primers bind.

Primer A: 5' ATATTCCCGCTCTCCGGAGA 3'

Primer B: 3' CTTACGCTTTGAGTCTCTAG 5'

As always when doing PCR, you furthermore need dNTPs (dATP, dTTP, dGTP og dCTP), a heat-stable DNA polymerase, salt and buffer. After for instance 100 PCR cycles, the mixture is run on an agarose gel next to a ladder containing DNA fragments of known size. Since only men have the SRY gene, a PCR product will only have been generated, if the DNA was taken from a man (otherwise there is no SRY gene and no place the primers can bind). The PCR product will be exactly 200 bp, which is the area between the two primers including the length of the two primers. If the athlete is a man, you will be able to see a band at 200 bp in the lane in which you put the mixture from the PCR. If the athlete is a woman, you will see no band in the lane in which you put the mixture.